

Sub
E1
D1
21. [Twice Amended] An isolated polynucleotide for enhancing protein expression, said polynucleotide comprising a nucleic acid sequence of nucleotides 181-341 of SEQ ID NO: 1 having one thymidine inserted between positions 206 and 207 of SEQ ID NO: 1, or a fragment thereof that includes said thymidine, wherein said polynucleotide or fragment enhances protein expression when incorporated downstream of an expression regulatory promoter sequence and upstream of a protein coding sequence.

22. [Twice Amended] The isolated polynucleotide according to claim 21, wherein said nucleic acid sequence has translation promoting activity to enhance expression of a nucleic acid sequence encoding a protein sequence.

23. [Twice Amended] The isolated polynucleotide according to claim 21, wherein said nucleic acid sequence enhances said expression by increasing IRES activity.

24. [Twice Amended] An isolated polynucleotide that enhances protein expression when included 5' of a protein coding sequence in an expression construct by promoting mRNA translation in an IRES dependent manner, said polynucleotide comprising a nucleotide sequence of SEQ ID NO: 7.

Please cancel claim 25 without prejudice.

Please amend claim 26 as follows.

D2
26. [Twice Amended] An isolated polynucleotide consisting of the nucleotide sequence as set forth in SEQ ID NO: 7 over its entire length.

Please cancel claim 27 without prejudice.

Please amend claims 28, 30, and 31 as follows.

D3
28. [Twice Amended] An expression vector comprising an isolated polynucleotide according to claim 21 or claim 24.

D4
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30. [Amended] A method of expressing a protein comprising the steps of:

- (a) transforming or transfecting a host cell with an expression vector according to claim 53,
- (b) growing the host cell in a medium under conditions where the cell expresses the protein.

31. [Amended] A method according to claim 30, further comprising a step of isolating the protein from the cell and/or the growth medium.

Please cancel claim 32, without prejudice.

Please amend claims 33 -39 as follows

33. [Amended] A probe for screening substances that interact with IRES, comprising the polynucleotide according to claim 26, further comprising a detectable label.

34. [Amended] A probe for screening IRES-dependent translation inhibitors, comprising the polynucleotide according to claim 26, further comprising a detectable label.

35. [Twice Amended] A composition comprising the isolated polynucleotide for enhancing protein expression according to claim 21.

36. [Amended] A composition comprising the isolated polynucleotide for enhancing protein expression according to claim 24.

37. [Twice Amended] A method for determining a hypervirulent hepatitis C strain, comprising the steps of:

(a) screening a biological sample for the presence of the polynucleotide according to claim 26, and;

(b) determining presence or absence of the hypervirulent hepatitis C strain from the screening step, wherein the presence of the polynucleotide identifies the hypervirulent hepatitis C strain in the biological sample and the absence of said sequence indicates the absence of said hypervirulent hepatitis C.

38. [Twice Amended] An isolated polynucleotide according to claim 21, further comprising nucleotides 1-180 of SEQ ID NO: 1.

39. [Twice Amended] An isolated polynucleotide according to claim 21 or 38, further comprising nucleotides 342-713 of SEQ ID NO: 1.

Please cancel claim 40-43, without prejudice.

Please amend claim 44 and 45 as follows.

44. [Twice Amended] An isolated polynucleotide comprising a nucleic acid sequence for enhancing expression of a nucleic acid sequence according to claim 24, wherein the 5'-untranslated region comprises a polynucleotide sequence corresponding to at least one region selected from the group consisting of pyrimidine-rich tract, Box A, Box B, a trans factor-binding site, and a combination thereof.

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45. [Twice Amended] An isolated polynucleotide comprising a nucleic acid sequence for enhancing expression of a nucleic acid sequence according to claim 44, wherein said nucleic acid comprises a sequence having substitution, deletion, insertion, and/or addition of a single or a few nucleotides of a sequence derived from a wild-type virus within the sequence or proximate sequence in at least one position corresponding to a pyrimidine-rich tract, Box A, Box B and/or trans factor-binding site contained in the 5'-untranslated region.

Please cancel claim 46, without prejudice.

Please add new claims 47-56.

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D7

47. [New] The isolated polynucleotide according to claim 24, wherein the 5'-untranslated region comprises at least one pyrimidine-rich tract.

48. [New] The isolated polynucleotide according to claim 24, wherein the 5'-untranslated region comprises a sequence corresponding to a region selected from the group consisting of Box A, Box B, a trans-binding site, and a combination thereof.

49. [New] The isolated polynucleotide according to claim 24, wherein the 5'-untranslated region comprises an AUG or ATG sequence.

50. [New] The isolated polynucleotide according to claim 24, wherein the 5'-untranslated region comprises a part of or an entire region of IRES of viral mRNA.

51. [New] The isolated polynucleotide according to claim 24, wherein said nucleic acid further comprises a portion of a coding region from a viral gene adjacent to the 5'-untranslated region.

52. [New] The isolated polynucleotide according to claim 24, wherein said nucleic acid is a cDNA sequence.

53. [New] An expression vector according to claim 28, further comprising a protein coding sequence operably inserted downstream of the polynucleotide for enhancing protein expression.

54. [New] An isolated polynucleotide comprising nucleotide 181-341 of SEQ ID NO: 1, wherein said polynucleotide includes a thymidine inserted between position 206 and 207 of SEQ ID NO: 1.